CONCISE COMMUNICATION

Resistance to β-lactams among blood isolates of *Salmonella* spp. in European hospitals: results from the SENTRY Antimicrobial Surveillance Program 1997–98

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The susceptibility to β-lactams and the β-lactamase content of 110 *Salmonella* spp. blood isolates collected during 1997–98 in 19 European centers participating in the SENTRY Surveillance Program were studied. Thirty-one isolates (28%) were resistant to penicillins, due to production of TEM-1 (27 isolates), OXA-1 (three isolates) or TEM-1 + OXA-1 (one isolate). All OXA-1 producers and 10 TEM-1-producing isolates were also resistant to penicillin–clavulanic acid combinations. In the latter isolates, this phenotype was associated with increased production of TEM-1. Sixteen TEM-1-producing *Salmonella Enteritidis* isolates and one OXA-1-producing *S. Typhimurium* isolate were able to transfer β-lactam resistance by conjugation.

Keywords *Salmonella*, β-lactams, resistance

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β-Lactam antibiotics, along with fluorinated quinolones, are the antibiotics of choice in the treatment of extraintestinal salmonella infections. A high proportion of *Salmonella* clinical strains produce common penicillinases such as TEM- and PSE-type enzymes [1,2]. There have also been recent studies reporting on the emergence of *Salmonella* strains producing various β-lactamases with a broad spectrum of activity that usually includes oxyimino-cephalosporins [3,4].

The aim of the SENTRY Antimicrobial Surveillance Program is to monitor isolation frequencies and antibiotic resistance trends of pathogenic microorganisms. This ongoing project operates through a worldwide network of sentinel hospitals. Analysis of data from European centers during 1998 showed that *Salmonella* ranks eleventh among the genera most frequently isolated from bloodstream infections [5]. In this study, we present the status of susceptibility to β-lactams and the respective resistance mechanisms of these *Salmonella* spp. isolates.

In total, 110 *Salmonella* spp. isolates derived from blood cultures during 1997–98 in 19 European centers were included in the study. Thirty-four were serotyped as *S. Enteritidis* and 21 as *S. Typhimurium*. Forty isolates were not characterized at the serotype level. The remaining isolates were distributed among various other serotypes. The isolates were collected for the SENTRY Program and deposited at the Eijkman-Winkler Institute for Microbiology, University Medical Center, Utrecht, The Netherlands. More details on the operation of this global surveillance system are given elsewhere [5,6]. Species identification was confirmed by the API 20E system (bioMerieux, Marcy l'Etoile, France). For selected isolates, serotyping results were confirmed at the National Reference Center for Salmonella and Shigella, School of Public Health, Athens, Greece (M. Lambiri and N. Vakalis, personal communication).

Susceptibility testing for β-lactams and other antimicrobials was performed by a broth microdilution method as recommended by the National

Committee for Clinical Laboratory Standards (NCCLS) [7]. Screening for production of extended-spectrum β-lactamases (ESBLs) was performed by the conventional double disk synergy test using an amoxicillin–clavulanate disk surrounded by cefotaxime, ceftazidime, aztreonam and cefepime disks at a distance of 30 mm (center to center). Occasionally, ESBL-detecting E test strips (AB Biodisk, Solna, Sweden) were used for confirmation.

Conjugal transfer of resistance to β-lactam antibiotics was performed in mixed broth cultures as described previously [4]. An *Escherichia coli* K12 strain highly resistant to rifampicin was used as recipient. Transconjugant clones were selected on nutrient agar containing ampicillin (50 mg/L) and rifampicin (200 mg/L). The frequency of transfer (transconjugants obtained per donor cell) was also estimated.

β-Lactamase preparations were obtained by ultrasonic treatment of bacterial cell suspensions. Extracts were clarified by centrifugation, and the protein content was determined with a protein assay kit (Bio-Rad, Munchen, Germany). Quantification of β-lactamase activity was done by spectrophotometry using the chromogen nitrocefin (Unipath, Basingstoke, UK) as substrate. Isoelectric points (pIs) of β-lactamases were determined by isoelectric focusing (IEF) in polyacrylamide gels containing ampholytes covering a pH range from 3.5 to 9.5 (Pharmacia-Biotech, Piscateway, NJ, USA). β-lactamases with known pIs (TEM-1, PSE-2, SHV-1 and SHV-5) were used as controls. β-Lactamase bands were visualized in situ with a nitrocefin solution.

PCR assays for the detection of *bla* genes were performed using oligonucleotide primers specific for the respective genes. For bla_{TEM} and bla_{PSE}, primers and PCR conditions as described by Llanes et al. were used [1]. For amplification of a segment of bla_{OXA-1}, primers 5′-GCATCCACAAACGCTGAAATTG-3′ (forward) and 5′-GAGCCATGCTTCTGTTAATCCG-3′ (reverse), encompassing nucleotides 1504–1852 of a published sequence (GenBank accession number J02967), were used.

Data are presented in Table 1. β-Lactam-resistant isolates were obtained by 13 of the 19 participating centers. In total, 31 of the 110 isolates (28%) exhibited high-level resistance to ampicillin; all were also resistant to ticarcillin. Fourteen were *S. Typhimurium* and 13 *S. Enteritidis*. The remaining four belonged to the serotypes Typhi, Dublin, Glostrup and Bredeney. Fifteen isolates (10 *S. Typhimurium* and five *S. Enteritidis*) were also resistant to piperacillin. Of the 31 ampicillin-resistant isolates, 13 (10 *S. Typhimurium* and three *S. Enteritidis*) were also resistant to at least one of the two penicillin–clavulanate combinations tested. All MICs of piperacillin–tazobactam were below the breakpoint for resistance (128/4 mg/L). Nevertheless, two *S. Typhimurium* isolates exhibited decreased susceptibility to this combination. Resistance to cefazolin was observed in three isolates. Cefotaxime, ceftriaxone, ceftazidime, aztreonam and cefepime were highly active against all 31 isolates (data not shown). There was no indication of production of ESBLs by the synergy tests employed.

In the IEF experiments, 27 isolates were found to produce a β-lactamase with a pI equal to 5.4. This enzyme was most probably TEM-1, since all 27 isolates were also positive in the bla_{TEM}-detecting PCR assays. Resistance to penicillins could be transferred from 17 of these isolates. Notably, conjugal transfer of TEM-1 was achieved with 12 *S. Enteritidis* but only two *S. Typhimurium* isolates. In addition, the frequency of transfer by *S. Enteritidis* was, on average, two orders of magnitude higher than that observed with *S. Typhimurium*. One *S. Enteritidis* and three *S. Typhimurium* isolates produced a β-lactamase with a pI of 7.4 and were also positive in the bla_{OXA-1} assays. A low-frequency conjugal transfer of OXA-1 was achieved with only one *S. Typhimurium* isolate (also producing TEM-1) which had been isolated in Albania.

The properties and relative quantities of β-lactamases produced may explain, at least in part, the differences in isolates' β-lactam resistance patterns. Comparison of specific activities against nitrocefin indicated that the penicillin-inhibitor-resistant isolates produced significantly higher quantities of TEM-1 than the susceptible isolates. Resistance to amoxicillin–clavulanate due to overproduction of TEM-1 in *Salmonella* has also been reported in previous studies [8]. This mechanism may also be associated with the high-level resistance to cefazolin observed in three of the isolates examined here. On the other hand, production of inhibitor-resistant TEM enzymes that also exhibit a pI of 5.4, such as TEM-39, -40 and -44, particularly among the penicillin-inhibitor-resistant, cefazolin-susceptible isolates, cannot be excluded. All four OXA-1 producers were resistant to at least one of

Table 1 Characteristics of 31 β-lactam-resistant *Salmonella* spp. clinical isolates

No	Serotype	Date of isolation	Place of isolation	β-Lactam resistance[a]	pI	PCR+ for	*bla* transfer
1	Typhimurium	01/98	Paris	A, T, P, Tc	5.4	*tem*	−
2	Typhimurium	03/98	Paris	A, T, P, Ac, Tc	5.4	*tem*	−
3	Typhimurium	05/97	Lille	A, T, P, Ac, (Tc)	5.4	*tem*	+
4	Typhimurium[b]	11/97	Lille	A, T, P, Ac	5.4	*tem*	−
5	Typhimurium	07/98	Lyon	A, T, P, (Tc), (Cf)	5.4	*tem*	−
6	Typhimurium[b]	11/97	Madrid	A, T, (P), Ac	7.4	*oxa-1*	−
7	Typhimurium[b]	04/98	Madrid	A, T, P, Ac, Tc	5.4	*tem*	−
8	Typhimurium	05/98	Barcelona	A, T, P, Ac, Tc, (Pz)	7.4	*oxa-1*	−
9	Typhimurium	07/98	Barcelona	A, T, P, Ac, Tc	5.4	*tem*	−
10	Typhimurium	04/98	Freiburg	A, T, P, Ac, Tc	5.4	*tem*	−
11	Typhimurium	04/98	Lausanne	A, T, (P)	5.4	*tem*	+
12	Typhimurium	05/98	Coimbra	A, T, (P)	5.4	*tem*	−
13	Typhimurium	10/98	Genova	A, T, (P)	5.4	*tem*	−
14	Typhimurium[b]	10/97	Tirana	A, T, P, Ac, Tc, (Pz), Cf	5.4 + 7.4	*tem* + *oxa-1*	+ (7.4)
15	Enteritidis[b]	09/98	Madrid	A, T, P, (Tc)	5.4	*tem*	+
16	Enteritidis	07/97	Barcelona	A, T, (P), Ac, Tc	7.4	*oxa-1*	−
17	Enteritidis	09/97	Barcelona	A, T, (P)	5.4	*tem*	+
18	Enteritidis	05/98	Barcelona	A, T, P	5.4	*tem*	+
19	Enteritidis	06/98	Barcelona	A, T, (P)	5.4	*tem*	+
20	Enteritidis	06/98	Barcelona	A, T, P	5.4	*tem*	+
21	Enteritidis	06/98	Barcelona	A, T, (P)	5.4	*tem*	+
22	Enteritidis	09/97	Sevilla	A, T, (P)	5.4	*tem*	+
23	Enteritidis	12/97	Sevilla	A, T, (P)	5.4	*tem*	+
24	Enteritidis	06/98	Sevilla	A, T, P, Ac, Tc	5.4	*tem*	+
25	Enteritidis	12/98	Sevilla	A, T, P, Ac, Tc, Cf	5.4	*tem*	+
26	Enteritidis	08/98	Athens	A, T, (P)	5.4	*tem*	+
27	Enteritidis[b]	08/98	Athens	A, T, (P)	5.4	*tem*	+
28	Typhi	07/97	Athens	A, T, (P)	5.4	*tem*	+
29	Dublin	10/97	Sevilla	A, T	5.4	*tem*	+
30	Glostrup[b]	04/97	Paris	A, T, P, (Tc), Cf	5.4	*tem*	−
31	Bredeney[b]	06/97	Athens	A, T, (P)	5.4	*tem*	+

[a]A, ampicillin; T, ticarcillin; P, piperacillin; Ac, amoxicillin–clavulanate; Tc, ticarcillin–clavulanate; Pz, piperacillin–tazobactam; Cf, cefazolin. Parentheses indicate intermediate susceptibility according to NCCLS criteria.
[b]Serotyped at the National Reference Center for Salmonella and Shigella, Athens, Greece.

the penicillin-inhibitor combinations tested. It is likely that this phenotype was determined by the relatively low sensitivity of OXA-1 to β-lactamase inhibitors as compared with TEM and other class A enzymes. Susceptibility of OXA-1 producers to cefazolin is in line with the weak activity of this β-lactamase against most narrow-spectrum cephalosporins [9].

In conclusion, the present study indicates that a considerable percentage of *S. Typhimurium* and *S. Enteritidis* strains isolated during 1997–98 from bloodstream infections in European hospitals were resistant to β-lactams, mostly due to the production of TEM-1. TEM-1 was also produced by an *S. Bredeney* isolate and an *S. Glostrup* isolate. To our knowledge, the presence of TEM in these serotypes has not been described before. The frequent occurrence of bla_{TEM} among salmonellae has also been observed in previous studies conducted in European countries [1,10]. On the other hand, OXA-type β-lactamases are encountered much less frequently than TEM among *Salmonella* isolates. Three of the four OXA-1-producing isolates in the present study were from Spain. This is consistent with a recent report on the isolation of *S. Typhimurium* with OXA-1 in this country [10]. Nevertheless, we cannot provide a plausible explanation for the absence of strains producing PSE-1, a frequent enzyme among multiresistant *S. Typhimurium* [1,2,10]. Notwithstanding the sampling limitations of sentinel surveys, it is expected that the SENTRY Program will efficiently monitor the rapid evolution of antimicrobial drug resistance trends in *Salmonella*.

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